Institute Report No. 325

Mutagenic Potential of 1-(1-Butyloxymethyl)-2-(E)-hydroxyiminomethyl-3-methylimidazolium Chloride in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test

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GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Mutagenic Potential of 1-(1-Butyloxymethyl)-2-(E)-hydroxyiminomethyl-3-methylimidazolium Chloride in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (Toxicology Series 125)--Sano and Korte

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The mutagenic potential of 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5.0 mg/plate to 0.0016 mg/plate. The test compound was not mutagenic under conditions of this test.

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ABSTRACT

The mutagenic potential of 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5.0 mg/plate to 0.0016 mg/plate. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE, Oxime

PREFACE

TYPE REPORT: Ames Test GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
Walter Reed Army Institute of Research
Washington, D.C. 20307-5100
Project Officer: H.A. Musallam

PROJECT/WORK UNIT/APC: 3M162734A875/308/TLEO

GLP STUDY NUMBER: 85007

STUDY DIRECTOR: MAJ Don W. Korte, Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: Steven K. Sano, BA, SGT, USA

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOPs, stability and purity data on the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE

INCLUSIVE STUDY DATES: 25 February 1985 - 22 March 1985

OBJECTIVE:

The objective of this study was to determine the mutagenic potential of 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (LAIR Code TP53) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

MAJ John W. Harbell, PhD, MSC, and Mr. John Dacey provided scientific guidance and research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85007 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr 30 NOV 88

DON W. KORTE JR, PhD / DATE
MAJ, MSC
Study Director

Steven Kazuo Sano 5 MAR 86

STEVEN K. SANO, BA / DATE
SGT, USA
Principal Investigator

Conrad Wheeler 14 July 88

CONRAD WHEELER, PhD/ DATE
DAC
Analytical chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 3 December 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Statement of Compliance

1. This is to certify that the protocol for GLP Study 85007 was reviewed on 21 February 1985.

2. The institute report entitled "Mutagenic Potential of 1-(1-Butyloxymethyl)-2-(E)-hydroxyiminomethyl-3-methylimidazolium Chloride in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 125, was audited on 14 November 1988.

Carolyn M. Lewis

CAROLYN M. LEWIS, MS
Diplomate, American Board of Toxicology
Chief, Quality Assurance

TABLE OF CONTENTS

Mutagenic Potential of 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test-- Sano and Korte

INTRODUCTION

1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was synthesized for a United States Army Medical Research and Development Command program charged with developing more effective oximes for treatment of nerve agent poisoning. The Ames Test is one of a series of tests in which these compounds will be evaluated to determine their relative potential for further development.

The Ames *Salmonella*/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of *Salmonella typhimurium* to detect compounds that are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating *in vivo* metabolic activation of the test compound. The Ames Test is an inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (1).

Objective of the Study

The objective of this study was to determine the mutagenic potential of 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (LAIR Code TP53) by using the revised Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Chemical Name: 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE

LAIR Code Number: TP53

Physical State: White crystalline solid

Source: SRI International, Menlo Park, CA

Storage: 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was received from SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025 and assigned the LAIR Code number TP53. The test compound was stored in a desiccator at 5°C until used.

Chemical Properties/Analysis: SRI International data characterizing the chemical composition and purity of the test material are presented in Appendix A along with confirmatory analysis of the test material performed by the Division of Toxicology, LAIR (Presidio of San Francisco, CA).

Test Solvent

The positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO). The test compound was dissolved in sterile deionized water obtained from a Polymetric model 200-3 Water Purifier (Sunnyvale, CA).

Chemical Preparation

On the day of dosing, 300 mg of the test compound was measured into a sterile vial and dissolved in 6 ml of sterile deionized water to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates.

Test Strains

Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538 obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory in liquid nitrogen. Quality control tests were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (2).

Test Format

1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was evaluated for mutagenic potential according to the methods of Ames *et al* (3). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (2).

Toxicity Tests:

Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE ranging from 1.6×10^{-3} mg/plate to 5 mg/plate, and approximately 10^8 cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin was placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth on the plates was observed. Since none of the plates showed a decreased number of macrocolonies (below the spontaneous rate) or an observable reduction in the density of the background lawn, the highest dose selected for the mutagenicity test was 5.0 mg/plate.

Mutagenicity Test:

The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5, both with and without 0.5 ml of the S-9 microsome fraction. The S-9 (batch R-315) was purchased from Litton Bionetics (Kensington, MD). The optimal titer of this S-9, as determined by Litton Bionetics, was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" concentrate (4). Plates were incubated upside down in the dark at 37°C for 48 hours. Plates were prepared in triplicate, and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Ames *et al* (3). Sterility and strain verification controls were run concurrently. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The integrity

of the different *Salmonella* strains used in the assay was verified by the following standard tests:

-Lack of growth (inhibition) in the presence of crystal violet which indicates that the prerequisite alteration of the lipopolysaccharide layer (LP) of the cell wall is present.

-Growth in the presence of ampicillin-impregnated disks which indicates the presence of an ampicillin-resistant R Factor in the TA98 and TA100 strains.

-Lack of growth (inhibition) following exposure to ultraviolet light which indicates the absence of the DNA excision-repair mechanism.

Four known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control but may be tested with several. These compounds (benzo[a]pyrene, 2-aminofluorene, 2-aminoanthracene, and N-methyl-N'-nitro-N-nitrosoguanidine) were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (5), a compound is considered mutagenic if a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count for the tester strains TA98 and TA100, or three times the spontaneous colony count for strains TA1535, TA1537, and TA1538. A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

Deviations from the Protocol/SOP

There were no deviations from the protocol or the standard operating procedures.

Storage of the Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

On 8 March 1985, the toxicity of 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was determined (Table 1). For this experiment all sterility, strain verification and negative controls were normal (Table 1). Exposure of the tester strain (TA100) to the highest dose showed neither a decrease in the number of macrocolonies nor an observable reduction in the density of the background lawn. Therefore, the highest dose selected for the mutagenicity test was 5.0 mg/plate. Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 11-14 March 1985 (Table 2). 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE did not induce an appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3). A tabular presentation of the raw data is included in Appendix B.

DISCUSSION

Certain test criteria must be satisfied before an Ames Test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, alterations in the LP layer, and deficiency in DNA excision-repair (except TA102). Second, the *Salmonella* strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on formation of macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of an Ames Test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was evaluated in the Ames Test. Criteria for a positive response include both a correlated dose response over three dose concentrations, and a revertant colony count at least two times (TA98, TA100) or three times (TA1535, TA1537, TA1538) the spontaneous revertant colony count (5). 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE did not induce the requisite dose-response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this test indicate that 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE is not mutagenic when evaluated in the Ames Test.

TABLE 1: TOXICITY LEVEL DETERMINATION FOR TP53

GLP STUDY NUMBER 85007

TOXICITY DETERMINATION REVERTANT PLATE COUNT (TA100)

CONCENTRATION	MEAN	±1SD	BACKGROUND LAWN*
5.0 mg/plate	115	4.7	NL
1.0 mg/plate	132	25.0	NL
0.2 mg/plate	110	28.4	NL
0.04 mg/plate	100	17.1	NL
0.008 mg/plate	109	9.8	NL
0.0016 mg/plate	105	20.0	NL

STRAIN VERIFICATION FOR TOXICITY DETERMINATION

	TA100*
HISTIDINE REQUIREMENT	NG
AMPICILLIN RESISTANCE	G
UV	NG
CRYSTAL VIOLET SENSITIVITY	NG
STERILITY CONTROL	NG

STERILITY CONTROL FOR TOXICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

*NL=Normal Lawn, G=Growth, NG=No Growth

TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING FOR THE MUTAGENICITY DETERMINATION OF TP53

GLP STUDY NUMBER 85007

STRAIN VERIFICATION

	OBSERVATIONS*				
STRAIN	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET	STERILITY CONTROL
TA98	NG	G	NG	NG	NG
TA100	NG	G	NG	NG	NG
TA1535	NG	NG	NG	NG	NG
TA1537	NG	NG	NG	NG	NG
TA1538	NG	NG	NG	NG	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG
S-9	NG

*G = Growth, NG = No Growth

TABLE 3: Mutagenicity Assay for 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP53)*

COMPOUND†	DOSE	TA98	TA100
		WITHOUT S-9	
NEG CONTROL	0.0 mg	17 ± 5.9	119 ± 16.7
MNNG	2.0 µg	- -	1802 ±305.5
MNNG	20.0 µg	- -	- -
TP53	5.0 mg	12 ± 2.3	114 ± 4.0
TP53	1.0 mg	13 ± 1.5	109 ± 9.0
TP53	0.2 mg	14 ± 4.5	111 ± 13.1
TP53	0.04 mg	16 ± 2.6	121 ± 7.9
TP53	0.008 mg	17 ± 2.3	109 ± 23.3
TP53	0.0016 mg	15 ± 1.5	83 ± 7.8
		WITH S-9	
NEG CONTROL	0.0 mg	20 ± 6.7	74 ± 15.2
AA	2.0 µg	418 ±132.1	575 ± 28.2
AF	2.0 µg	353 ± 64.4	137 ± 9.8
BP	2.0 µg	240 ± 43.5	164 ± 19.1
TP53	5.0 mg	27 ± 2.3	95 ± 6.0
TP53	1.0 mg	25 ± 3.1	82 ± 12.5
TP53	0.2 mg	19 ± 9.0	70 ± 8.5
TP53	0.04 mg	21 ± 4.0	85 ± 16.0
TP53	0.008 mg	20 ± 1.2	72 ± 12.5
TP53	0.0016 mg	25 ± 4.2	55 ± 11.1

*Values represent the mean number of revertants/plate (± standard deviation)
†MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

TABLE 3 (cont.): Mutagenicity Assay for 1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP53)*

COMPOUND†	DOSE/PLATE	TA1535	TA1537	TA1538
		WITHOUT S-9		
NEG CONTROL	0.0 mg	39 ± 6.0	6 ± 2.4	14 ± 2.5
MNNG	2.0 µg	- -	- -	- -
MNNG	20.0 µg	1798 ±255.1	- -	- -
TP53	5.0 mg	38 ± 6.4	4 ± 1.0	12 ± 2.3
TP53	1.0 mg	31 ± 1.5	4 ± 1.2	13 ± 4.7
TP53	0.2 mg	34 ± 3.5	3 ± 0.6	12 ± 3.2
TP53	0.04 mg	31 ± 6.5	4 ± 1.0	17 ± 2.1
TP53	0.008 mg	34 ± 6.8	3 ± 1.2	12 ± 0.6
TP53	0.0016 mg	28 ± 5.9	5 ± 1.5	11 ± 1.2
		WITH S-9		
NEG CONTROL	0.0 mg	27 ± 17.1	6 ± 2.7	19 ± 6.6
AA	2.0 µg	- -	164 ±88.0	549 ±54.5
AF	2.0 µg	- -	- -	320 ±62.6
BP	2.0 µg	- -	44 ±13.1	102 ±10.1
TP53	5.0 mg	17 ± 4.9	4 ± 2.0	23 ± 1.0
TP53	1.0 mg	13 ± 1.7	4 ± 1.5	21 ± 3.5
TP53	0.2 mg	14 ± 3.6	5 ± 2.1	24 ± 3.5
TP53	0.04 mg	16 ± 1.0	5 ± 1.0	25 ± 6.0
TP53	0.008 mg	17 ± 1.7	4 ± 1.0	18 ± 2.6
TP53	0.0016 mg	12 ± 2.3	5 ± 1.2	20 ± 3.5

*Values represent the mean number of revertants/plate (± standard deviation)
†MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, AA=2- aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

CONCLUSION

1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE was evaluated for mutagenic potential in the Ames Test, in both the presence and the absence of metabolic activation, and did not induce a positive mutagenic response under conditions of this study.

REFERENCES

1. McCann J, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the *Salmonella*/Mammalian Microsome Mutagenicity Test: Test of 300 chemicals. Proc Nat Acad Sci, USA 1975;72:5135-5139.

2. Ames *Salmonella*/Mammalian Microsome Mutagenesis Test. LAIR Standard Operating Procedure OP-STX-1, Presidio of San Francisco, California: Letterman Army Institute of Research, 29 August 1986.

3. Ames BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with Salmonella/Mammalian Microsome Mutagenicity Test. Mutat Res 1975;31:347-364.

Vogel HJ, Bonner DM. Acetylornithinase of *E. coli*: Partial purification and some properties. J Biol Chem 1956;218:97-106.

5. Brusick D. Genetic toxicology. In: Hayes AW, ed. Principles and methods of toxicology. New York: Raven Press, 1982:223-272.

APPENDICES

APPENDIX A: Chemical Data

Chemical Name: 1-(butoxymethyl)-2-((hydroxyimino)methyl)-3-methyl-1H-imidazolium chloride

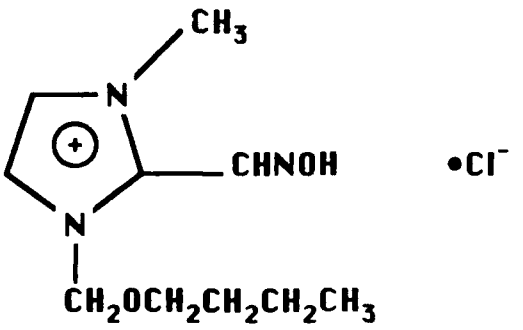
Alternate Chemical Names:

1-(1-Butyloxymethyl)-2-(E)-hydroxyiminomethyl-3-methylimidazolium chloride,
1-(1-Butoxymethyl)-2-[(hydroxyimino)methyl]-3-methylimidazolium chloride

Chemical Abstracts Service Registry Number: 91900-13-9

LAIR Code Number: TP53

Chemical Structure:



Molecular Formula: $C_{10}H_{18}ClN_3O_2$

Molecular Weight: 247.5

Source: Clifford D. Bedford, PhD
SRI International, Physical Sciences Division
Menlo Park, CA

SRI Reference Number: BHH-0016

APPENDIX A (cont.): Chemical Data

Analytical Data: Data supplied by SRI International included melting point, elemental analysis, and NMR and IR spectra.[1] Melting point: 100-103°C . Elemental analysis calculated for $C_{10}H_{18}ClN_3O_2$: C, 48.49; H, 7.32; N, 16.96; Cl, 14.31. Found: C, 48.23; H, 7.51; N, 17.09; Cl, 14.51. NMR (60 MHz, d_6DMSO) δ 0.70-1.70 (br m, 7H, alkyl), 3.53 (t, 2H, J= 6.0 Hz, CH_2), 4.05 (S, 3H, CH_3), 5.87 (S, 2H, CH_2), 8.18 (d, 1H, J= 2.0 Hz, aryl), 8.30 (d, 1H, J= 2.0 Hz, aryl), 8.63 (S, 1H, CH), 13.53 (S, 1H, NOH). IR (KBr) 2900, 1575, 1510, 1420, 1380, 1280, 1205, 1115, 1060, 995, 860, 750 cm^{-1}. The IR spectrum obtained upon receipt of the compound confirmed the identity of the material.[2]

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[1] Bedford CD. Notebook reference 5851-74. Stanford Research International, Physical Sciences Division, Menlo Park, CA.

[2] Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p21. Letterman Army Institute of Research, Presidio of San Francisco, CA.

APPENDIX B: Individual Plate Scores

1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP53)

TOXICITY DETERMINATION WITH TA100

DOSE/PLATE	5.0 mg	1.0 mg	0.2 mg	0.04 mg
PLATE 1	113	103	105	119
PLATE 2	120	143	85	86
PLATE 3	111	149	141	95
background lawn	NL*	NL	NL	NL

DOSE/PLATE	0.008 mg	0.0016 mg	NEG CONTROL
PLATE 1	106	84	114
PLATE 2	101	124	123
PLATE 3	120	106	125
background lawn	NL	NL	NL

* NL=Normal Lawn

APPENDIX B (cont.): Individual Plate Scores

1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP53)

NEGATIVE CONTROL DATA

COMPOUND	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
			WITHOUT S-9			
NEG CONTROL (START RUN)	0.0 mg	21	110	44	5	15
		17	129	39	3	13
		10	99	48	9	10
NEG CONTROL (END RUN)	0.0 mg	16	142	32	4	12
		12	130	36	7	15
		26	106	35	8	17
			WITH S-9			
NEG CONTROL (START RUN)	0.0 mg	26	69	47	2	14
		25	64	30	6	12
		8	55	47	5	13
NEG CONTROL (END RUN)	0.0 mg	21	86	8	8	24
		19	74	16	10	25
		24	97	14	6	26

APPENDIX B (cont.): Individual Plate Scores

1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP53)

POSITIVE CONTROL DATA

COMPOUND†	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
AA	2.0 µg	294	555		266	585
		403	562		114	486
		557	607		113	575
AF	2.0 µg	280	129			367
		402	148			344
		377	134			249
BP	2.0 µg	261	170		38	93
		190	180		59	113
		269	143		35	101
MNNG	2.0 µg		1521			
			1757			
			2127			
MNNG	20.0 µg			2063		
				1778		
				1554		

†AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine

APPENDIX B (cont.): Individual Plate Scores

1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP53)

MUTAGENICITY DATA WITHOUT S-9

COMPOUND	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
TP53	5.0 mg	11	118	45	3	9
		15	114	33	4	13
		11	110	35	5	13
TP53	1.0 mg	13	109	31	5	18
		12	100	29	3	9
		15	118	32	3	11
TP53	0.2 mg	14	96	38	3	14
		19	120	31	4	13
		10	117	34	3	8
TP53	0.04 mg	15	115	31	5	19
		19	130	38	4	15
		14	118	25	3	16
TP53	0.008 mg	18	102	32	2	11
		14	90	42	4	12
		18	135	29	2	12
TP53	0.0016 mg	16	74	21	5	10
		13	85	32	6	12
		15	89	30	3	12

APPENDIX B (cont.): Individual Plate Scores

1-(1-BUTYLOXYMETHYL)-2-(E)-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE (TP53)

MUTAGENICITY DATA WITH S-9

COMPOUND	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
TP53	5.0 mg	30	89	14	6	24
		26	96	23	2	22
		26	101	15	4	23
TP53	1.0 mg	24	94	12	4	24
		28	83	15	5	21
		22	69	12	2	17
TP53	0.2 mg	19	70	17	7	26
		10	62	10	3	20
		28	79	15	6	26
TP53	0.04 mg	19	86	17	4	31
		26	100	16	5	19
		19	68	15	6	25
TP53	0.008 mg	21	71	16	5	21
		19	85	19	3	16
		21	60	16	4	17
TP53	0.0016 mg	22	53	9	4	16
		24	45	13	4	23
		30	67	13	6	20

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